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Karyotype relationship among four species of Spiny mice (*Acomys*, Rodentia)

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Abstract

G-banded karyotypes of four species of the genus *Acomys* from different localities are described: *Acomys cahirinus* from Egypt, *A. cineraceus* from the Sudan, *A. dimidiatus* from Israel and *A. minous* from Crete. Diploid chromosome numbers vary considerably among these species, ranging from 2n = 36 to 2n = 50. The variation is due to Robertsonian fusions and/or fissions. We constructed a maximum parsimony tree using the common Robertsonian metacentrics as characters.

Key words: *Acomys*, karyotype, Robertsonian chromosome, phylogeny

Introduction

The genus *Acomys* (spiny mice) has a wide geographical distribution from South Africa to southwest Asia and as far north as Crete and Turkey. Preferring rocky habitats, *Acomys* displays a patchy distribution with many populations isolated on cliffs and rocky hills (Volobouev et al. 1991). Currently, 52 taxa are assigned to the genus *Acomys*. They have been assembled into a variety of subgenera, species groups, and species (Bates 1994). Due to the absence of unequivocal diagnostic markers, the taxonomic classification changed several times. The proposed number of species ranges from 14 (Musser and Carleton 1993) to 38 (Ellermann 1941). We follow the classification of Denys et al. (1994) who gave diagnoses based on skull morphology, dental pattern, allozyme patterns, and karyotypes.

Volobouev et al. (1991, 1996 a, b) applied cytotaxonomy to clarify the confusing systematics of this group. Karyotypes display a wide variation among species. Diploid chromosome numbers from 2n = 36 to 2n = 68 have been found. The variation is presumed to be mainly due to centric fusion and fission events (Robertsonian translocations).

In this study we describe the G-band karyotypes of four species of *Acomys* and draw phylogenetic conclusions from the presence of common Robertsonian (Rb) chromosomes.

Material and methods

Animals

Our specimen of *A. minous* (Bate, 1905) was from Crete. Specimens from *A. cahirinus* (Desmarest, 1819) were kindly provided by the Zoological Garden of Cairo. Animals originally derived from Abu Rawash (Egypt) had yellow coat colour, those from Kardasa (Egypt) were grey. *A. cineraceus*

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(Fitzinger and Heuglin, 1866) was from two sources: the Blue Nile province (Sudan) and the province of Kordofan (Sudan). The Kordofan specimens were kindly provided by the late Dr. Jochen Niethammer (Bonn). *A. dimidiatus* (Cretzschmar, 1826) was trapped in Jerusalem by the late Dr. Alfred Gropp (Lübeck) and by Dr. Jacob Wahrman (Jerusalem). The *A. minous* (♀)×*A. dimidiatus* (♂) hybrid had been bred and kindly supplied by Dr. Jacob Wahrmann.

Mitotic chromosomes

Metaphase chromosomes were G-banded according to Seabright (1971). At least 10 metaphases from each species were analysed.

Meiotic chromosomes

The ovaries of the hybrid animal were dissected out and placed into cell culture medium (TC199). Oocytes were released by puncturing mature follicles with fine needles. The oocytes were collected and transferred into a petri dish with fetal calf serum. They were incubated at 36 °C for about six hours, then in 1 % Na-citrate for about 15 minutes. After transfer to clean slides in a drop of hypotonic solution, spreading and fixation were achieved by dropping methanol : acetic acid (3 : 1) onto the cells. Chromosomes were stained with orcein.

Results and discussion

Karyotypes

The *A. cahirinus* specimens had 18 chromosome pairs (2n = 36), 16 of which were metacentric (Fig. 1). Only the smallest pair of autosomes (no. 17) was acrocentric. Karyotypes of individuals from Abu Rawash and Kardasa were identical. The karyotypes are in keeping with the R-band karyotype published by Volobouev (1996 b).

The *A. minous* complement consisted of 19 chromosome pairs (2n = 38). Of the autosome pairs, 15 were metacentric and three acrocentric (Fig. 2). According to Matthey (1963), the *A. minous* karyotype is polymorphic. It contains 14 pairs of metacentrics and either four or five pairs of acrocentric autosomes. Our *A. minous* karyotype may have been derived from the latter one by a Robertsonian fusion (or the latter one from our karyotype by a fission event).

A. cineraceus from the Blue Nile province (Sudan) was polymorphic. The chromosome complement consisted of 25 pairs (2n = 50) with nine metacentric ones (Fig. 3) or of 24 chromosome pairs (2n = 48), among which ten were metacentric. One of the latter metacentrics was related to the acrocentric chromosomes no. 16 and no. 18 (indicated with asterisks in Fig. 3) by a Robertsonian fission/fusion event. The chromosome complement of specimen from Kordofan was identical to the 2n = 48 karyotype.

The *A. dimidiatus* specimen from Israel had 19 chromosome pairs (2n = 38), 16 of which were metacentric (Fig. 4). The same number of chromosomes was found in *A. cf. dimidiatus* from Saudi Arabia by Volobouev et al. (1991).

The sex chromosomes of all four *Acomys* species were acrocentric.

Common Robertsonian metacentrics

To investigate the phylogenetic relationship, we compared the mitotic karyotypes of the four species. Single arm comparisons proved unsatisfactory as the low number of bands did not allow safe identification. We were in a better position with whole Robertsonian metacentrics (Rbs). They afforded the combined banding pattern of the two arms and the

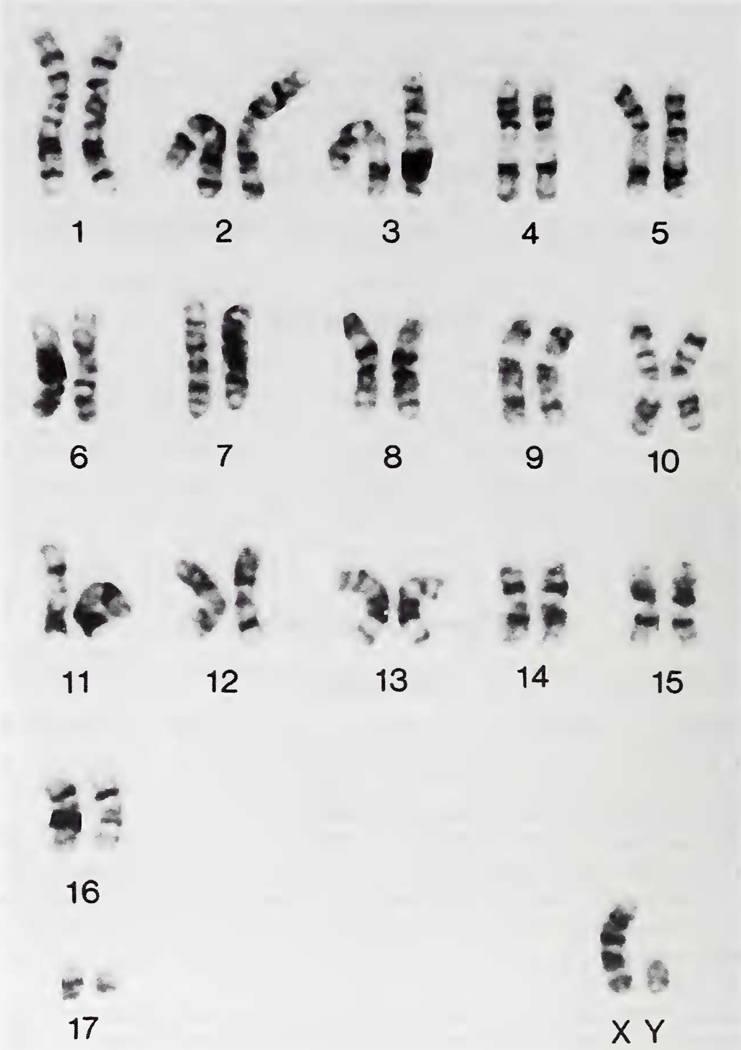

Fig. 1. G-band karyotype of *Acomys cahirinus* ♂ from Abu Rawash. Chromosomes were arranged according to size within each of three groups, metacentric autosomes, acrocentric autosomes and sex chromosomes.

centromere position as characters for identification. We focussed, therefore, on the recognition of common Rbs among the four species.

Several Rbs common to two or more species have been identified (Tab. 1). The karyotypes of *A. cahirinus* and *A. minous* had 15 Rbs in common (Tab. 1: A–O). The arms of *A. cahirinus* Rb chromosome no. 1 had their counterparts in the *A. minous* acrocentrics no. 16 and no. 17. Three Rbs (D, L, O; Fig. 5, Tab. 1) from *A. cahirinus* were found in *A. minous* and *A. cineraceus*, another Rb (G; Fig. 5, Tab. 1) in *A. cahirinus*, *A. minous*, and *A. dimidiatus*.

Our identification of one common Rb in *A. minous* and *A. dimidiatus* was supported by diakinesis figures from an *A. minous* (♀) × *A. dimidiatus* (♂) hybrid. The ooyctes of

Fig. 2. G-band karyotype of *A. minous* ♂. Arrangement of chromosomes as in Fig. 1.

the hybrid displayed three bivalents and two multivalent chains (Fig. 6). Most probably, the smallest bivalent (Fig. 6, arrowhead) consists of the paired acrocentrics no. 18 from *A. minous* and no. 18 from *A. dimidiatus* while the larger ring bivalents (Fig. 6, arrows) are the X chromosome bivalent and the bivalent of the only common Rb (G, Tab. 1). The two multivalent chains comprise all remaining chromosomes. Such synaptic chains are well known from hybrids between different Rb chromosome races, e. g., from *Mus musculus* (Johannisson and Winking 1994). They are characteristic for pairing of Rbs with alternating arm composition. The two chains in the *Acomys* hybrid are, therefore, evidence for the presence of Rbs with non-homologous arm composition in the parent species. The chains are terminated at their ends by 3 acrocentrics (two from *A. minous* and one from *A. dimidiatus*) and one small *A. dimidiatus* metacentric chromosome which, according to Volobouev et al. (1996 a) originated from an acrocentric one by an inversion. Hence, the pairing figures are fully accounted for.

Matthey (1963) published a meiosis figure from a hybrid which was designated *A. cahirinus* (♀) × *A. minous* (♂) but which according to the classification of Al-Saleh (1988) is to be considered an *A. dimidiatus* (♀) × *A. minous* (♂) hybrid. Thus, this is in

Fig. 3. G-band karyotype of *A. cineraceus* ♂ from Blue Nile. Arrangement of chromosomes as in Fig. 1.

fact the reciprocal hybrid to the one described by us and it confirms the conclusions drawn. The only apparent difference is the formation of three instead of two multivalent chains. This agrees with the presence of five instead of three acrocentric autosomes in the *A. minous* parent karyotype.

In contrast to us, VOLOBOUEV et al. (1996 b) did not find a common Rb in the complements of *A. cahirinus* and *A. dimidiatus*. This may have been due to a difference between populations. The animal investigated by VOLOBOUEV et al. (1996 b) came from Saudi Arabia, while our *A. dimidiatus* specimen was from Israel.

Fig. 4. G-band karyotype of *A. dimidiatus* ♀. Arrangement of chromosomes as in Fig. 1.

Karyotype evolution

The presence of common Rbs (Tab. 1) was translated to a maximum parsimony tree (Fig. 7). The only inconsistant character with respect to the tree topology is Rb G.

This Rb was either present at the root of the tree and lost in the *A. cineraceus* branch (Fig. 7 a) or it was independently created by fusion in the *A. dimidiatus* and in the *A. cahirinus/A. minous* branch (Fig. 7 b). We favour the interpretation represented in figure 7 a. The presence of a high number of chromosomes considered, the probability to create the same Rb in independent translocation events is low.

According to our dendrogram *A. cahirinus* and *A. minous* are the closest relatives among the four species. According to morphological parameters *A. minous* was treated as a separate species which belongs to the *A. cahirinus-A. dimidiatus* group (Corbet and Hill 1991; Denys et al. 1994; Dieterlen 1978). Morphologically *A. cahirinus* and

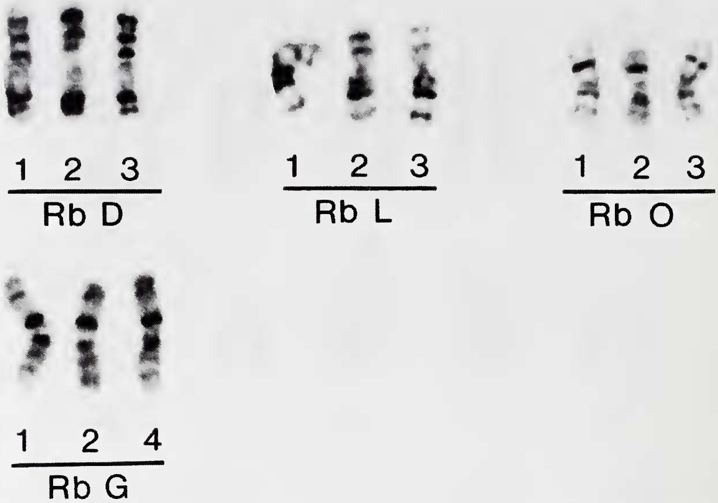

Fig. 5. Common Rb chromosomes in three *Acomys* species. Designation of Rbs according to table 1. Chromosomes from *A. cahirinus* (1), *A. minous* (2), *A. cineraceus* (3), and *A. dimidiatus* (4).

Table 1. Rb chromosomes common to at least two *Acomys* species (numbering of chromosomes according to figures 1–4).

A. cahirinus no.	*A. minous* no.	*A. cineraceus* no.	*A. dimidiatus* no.	Designation
2	1			A
3	2			B
4	3			C
5	4	3		D
6	5			E
7	6			F
8	7		9	G
9	8			H
10	9			I
11	10			J
12	11			K
13	12	7		L
14	13			M
15	14			N
16	15	9		O

A. dimidiatus appear to be the closest relatives, they are nearly indistinguishable (Volobouev et al. 1996 b). The distance of *A. cahirinus* and *A. dimidiatus* is confirmed, however, by a comparison of satellite DNA sequences (Kunze et al. 1999).

The evolutionary history of the genus *Acomys* is not well documented, but the starting point appears to be tropical Africa with the oldest fossil records being about 7–8 my old (Denys et al. 1994). *Acomys* species with different Rbs were assumed by Volobouev et al. (1996 a) to be derived from a common ancestor with a karyotye of 68–70 acrocentric chromosomes. Denys et al. (1994) assumed that this hypothetic common ancestor spread slowly over the continent and reached North Africa about 120 000 years ago and

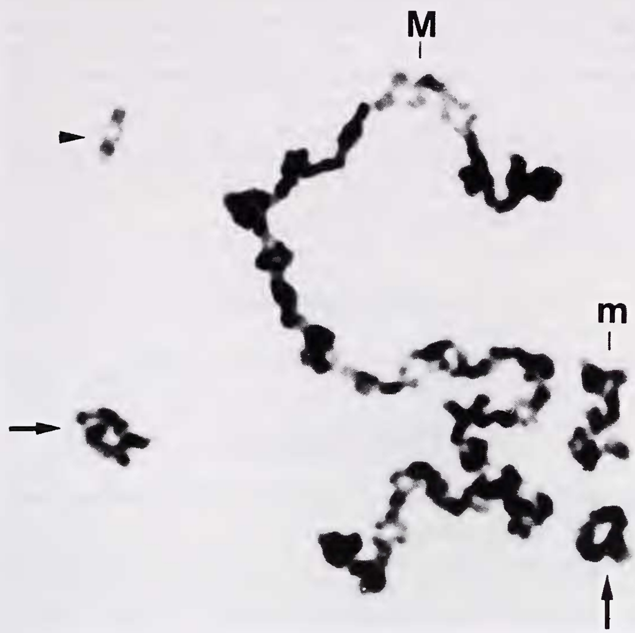

Fig. 6. Diakinesis figure of an *A. minous* (♀) × *A. dimidiatus* (♂) female hybrid. Arrowhead: bivalent of two acrocentric autosomes; arrows: ring bivalents of X/X and Rb G; m: short multivalent chain; M: long multivalent chain.

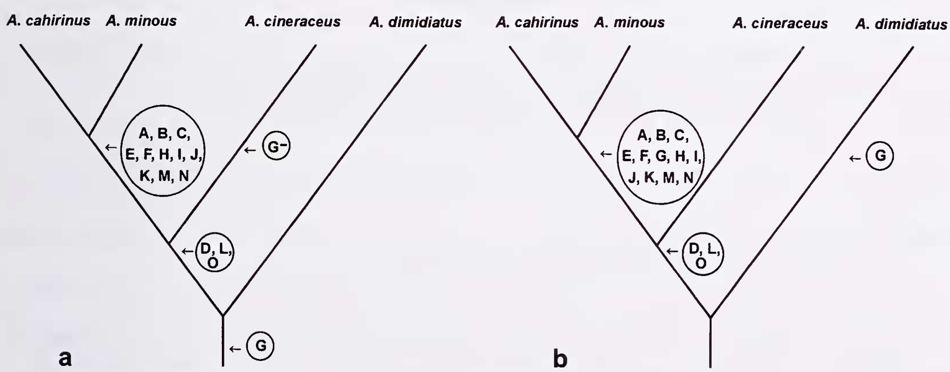

Fig. 7. Maximum parsimony tree, based on common Rbs. a) and b) differ with respect to the interpretation of G. Fusion events are indicated by resulting Rbs (A, B...O). Fission of Rb G is indicated by G^{-}.

appeared in Israel about 40 000 years ago (Tchernov 1968). The Cretan population was assumed to be founded by an ancestor with an all acrocentric karyotype (Denys et al. 1994).

From the chromosome tree of the four species we infer a different scenario of karyotype diversification. A common ancestor with a nearly all acrocentric karyotype – it probably contained Rb G – invaded Egypt about 120 000 and Israel about 40 000 years ago.

Waves of Robertsonian fusion started independently in Northern Africa and Israel. They led to a nearly all-metacentric state in the two species *A. cahirinus* and *A. dimidia-*

tus. Since the karyotypes from the Cretan species *A. minous* is very similar to that of the Egyptian species *A. cahirinus* we assume that Crete was populated by Egyptian *Acomys* after fixation of most Rb chromosomes. *Acomys* may have reached Crete by ship with humans. This assumption is in accordance with the lack of fossil *Acomys* records in Crete (Dieterlen 1978).

A corollary of this scenario is that Rb formation and fixation was a rapid process, at least in the Israelian spiny mice. The rate of Rb fixation (15 Rbs in 40 000 years) is, however, in accordance with estimates by Ferris et al. (1983). They estimated the rate of Rb fixation in *Mus musculus* to be 1 per 1 000 years.

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Zusammenfassung

Karyotypische Verwandtschaftsbeziehungen zwischen vier Arten der Gattung Acomys (Rodentia)

In dieser Arbeit werden G-Banden Karyotypen von vier verschiedenen *Acomys* Arten aus verschiedenen Regionen beschrieben: *Acomys cahirinus* aus Ägypten, *A. cineraceus* aus dem Sudan, *A. dimidiatus* aus Israel und *A. minous* von Kreta. Die diploide Chromosomenzahl variiert erheblich zwischen diesen Arten und reicht von 2n = 36 bis 2n = 50. Die Unterschiede werden im Wesentlichen durch Robertson-Translokationen verursacht. Wir benutzen das Auftreten von gemeinsamen Robertson-Chromosomen in verschiedenen Arten als Merkmale zur Konstruktion eines Stammbaums.

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